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TO: Assistant Commissioner for Patents
Box Patent Applications
Washington D.C. 20231

Attorney Docket No.017283/0123
(must include alphanumeric codes if no inventors named)

UTILITY PATENT APPLICATION TRANSMITTAL
(new nonprovisional applications under 37 CFR 1.53(b))

Transmitted herewith for filing is the patent application of:

INVENTOR(S): Patrick BENOIT; Francois MEYER; Debborah MAGUIRE; Ivan PLAVEC; and Michael G. TOVEY

TITLE: MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

In connection with this application, the following are enclosed:

APPLICATION ELEMENTS:

XX Specification - 30 TOTAL PAGES

(preferred arrangement:)

-Descriptive Title of the Invention
-Cross Reference to Related Applications
-Statement Regard Fed sponsored R&D
-Reference to Microfiche Appendix
-Background of the Invention
-Brief Summary of the Invention
-Brief Description of the Drawings (if filed)
-Detailed Description
-Claim(s)
-Abstract of the Disclosure

XX Drawings - Total Sheets 5

XX Declaration and Power of Attorney - Total Sheets 4

____ Newly executed (original or copy)

XX Copy from a prior application (37 CFR 1.63(d))

(relates to continuation/divisional boxes completed) - NOTE: Box below

____ DELETION OF INVENTOR(S) - Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

XX Incorporation By Reference (useable if copy of prior application Declaration being submitted)

The entire disclosure of the prior application, from which a COPY of the oath or declaration is supplied as noted above, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

____ Microfiche Computer Program (Appendix)

XX Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)

____ Computer Readable Copy
XX Paper Copy (identical to computer copy)
____ Statement verifying identify of above copies

ACCOMPANYING APPLICATION PARTS

____ Assignment Papers (cover sheet & document(s))
____ 37 CFR 3.73(b) Statement (when there is an assignee)
____ English Translation Document (if applicable)
_XX_ Information Disclosure Statement(IDS) with PTO-1449
_XX_ Preliminary Amendment
_XX_ Return Receipt Postcard (MPEP 503)
____ Small Entity Statement(s)
____ Statement file in prior application, status still proper and desired.
____ Certified Copy of Priority Document(s) with Claim of Priority (if foreign priority is claimed).
_XX_ OTHER: Check in the amount of $760.00

If a **CONTINUING APPLICATION, check appropriate box and supply the requisite information:**
____ Continuation _XX_ Divisional ____ Continuation-in-part (CIP) of prior application Serial No. 08/307,588 .

_XX_ Amend the specification by inserting before the first line the following sentence: --This application is a ____ continuation, _XX_ divisional or ____ continuation-in-part of application Serial No. 08/307,588 , filed December 5, 1994 , which is in turn a National Stage of International Application PCT/EP93/00770, filed March 30, 1993--.

CORRESPONDENCE ADDRESS:

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FEE CALCULATIONS: (Small entity fees indicated in parentheses.)

(1) For	(2) Number Filed	(3) Number Extra	(4) Rate	(5) Basic Fee $760 ($380)
Total Claims	5 - 20 =	0	x $18 (x $9)	
Independent Claims	2 - 3 =	0	x $78 (x $39)	
Assignment Recording Fee per property			$40	
			TOTAL FEE:	$760.00

METHOD OF PAYMENT:

A check in the amount of the above TOTAL FEE is attached. If payment is enclosed, this amount is believed to be correct; however, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 19-0741.

Respectfully submitted,

Date: February 2, 1999
Docket No.: 017283/0123

Bernhard D. Saxe
Reg. No. 28,665

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 017283/0123

In re patent application of

Patrick BENOIT *et al.*

Serial No.: Unassigned

Filed: February 2, 1999

For: MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered:

IN THE SPECIFICATION:

Page 8, line 14, after "figure 3," insert --(SEQ ID NOS: 3-4)--.

Page 8, lines 18 and 25, after "figure 2," insert --(SEQ ID NOS: 1-2)--.

Page 8, line 27, after "229," insert --of SEQ ID NO: 1 or 2--.

Page 10, line 12, insert --is-- between "it" and "directed".

Page 10, line 16, after "figure 2," insert __SEQ ID NOS: 1-2)--.

Page 13, line 15, after "sequence" insert --(SEQ ID NOS: 1-2)--.

Page 13, line 23, after "sequence" insert --(SEQ ID NOS: 3-4)--.

Page 14, line 7, after "figure 2," insert --; SEQ ID NOS: 1-2--.

Page 24, at the end of the specification, before the claims, insert the printed Sequence Listing submitted concurrently herewith, and renumber pages 1-9 of the Sequence Listing as pages 25-33 of the specification.

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

--23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605).

24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.

25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.

26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.

27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.

28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT

Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999
Date

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ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R), which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFNα and IFNβ together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFNα locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, αII or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFNα genes (Weissmann and Weber 1986).

The subtypes of IFNα have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFNβ) is encoded by a single gene which has approximately 50% homology with the IFNα genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFNα and IFNβ genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFNα, and type I-IFN will represent both natural IFNα, IFNω, and IFNβ.

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immuno-regulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class 1 antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVDH. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVDH in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β)is abnormaly produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leucocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α, β, ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellar domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monocolonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 µg/ml, preferably equal or inferior to 50 µg/ml, advantageously inferior to 20 µg/ml, more preferably in the range of approximately 0.5 to 2 µg/ml.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 µg/ml.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 µg/ml.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 µg/ml, preferably 1 to 20 µg/ml, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁻ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antobodies as defined above, together with an appropriate pharmaceutical vehicle.

The invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, asplatic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I-labelled monoclonal antibodies 34F10 and 64G12 to :
  - A : Daudi cells
  - B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was mesured by incubation with a 100 fold exces of cold antibodies and substracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthetized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

E X A M P L E S

EXAMPLE 1 :

Synthesis of the soluble receptors
Synthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yieled a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)
|
|
IPTG induction 3h
|
|
cell pellet
6M Guanidine hydrochloride pH8
|
|
centrifugation
|
|
NTA column: Washes pH 8 urea 8M
| pH 6,3 urea 8M
| pH 5.9 urea 8M
Elution pH 4 urea 8M
|
|
refolding dilution, dialysis
| against Tris 0,1 M pH9
|
dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromotography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells
|
| 18 h
|
serum free medium
|
|
supernatants taken after 48h, 72h, 96h
|
|
concentration
|
|
NTA column
|
| Wash PBS
elution 0.1 M NaOAc pH 5.5
|
| neutralization
concentration, 30 000 cut off
|
|
Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10µg of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT$^-$ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, $5x10^7$ spleen cells were fused to $3x10^7$ myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as $20x10^7$ spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, $5x10^5$ Daudi cells were incubated in 100µl of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse $F(ab')_2$ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :

Reactivity of the anti IFN-R monoclonal antibodies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		
	ELISA	Western	ELISA	Western	immunofluorescence
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+ weak	+

* measured on Daudi cells

EXAMPLE 3 :

Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I-labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Researh, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4µg/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11µg/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60µg/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAB64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 µg/ml
HL60	11 µg/ml
Ly28	60 µg/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ^{125}I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 µg/ml, a high affinity binding of the mAb 34F10 (≈10nM) was seen on all cell lines whereas a high affinity binding of mAB 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells (0.5×10^6/ml) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C. Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :
   - it recognizes the extracellular domain of the human IFN-R, and
   - it has a neutralizing capacity against the biological properties of the human type I-IFN.
2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.
3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.
4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.
5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 µg/ml, preferably equal or inferior to 50 µg/ml, advantageously inferior to 20 µg/ml, more preferably in the range of approximately 0,5 to 2 µg/ml.
6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 µg/ml.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 µg/ml.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 µg/ml.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 µg/ml.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 ~~to 11~~, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: BENOIT, Patrick
MEYER, Francois
MAGUIRE, Deborah
PLAVEC, Ivan
TOVEY, Michael G.

(ii) TITLE OF INVENTION: MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Foley & Lardner
(B) STREET: 3000 K Street, N.W., Suite 500
(C) CITY: Washington
(D) STATE: D.C.
(E) ZIP: 20007

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/307,588
(B) FILING DATE: 05-DEC-1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/EP93/00770
(B) FILING DATE: 30-MAR-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 92400902.0
(B) FILING DATE: 31-MAR-1992

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: SAXE, Bernhard D.
(B) REGISTRATION NUMBER: 28,665
(C) REFERENCE/DOCKET NUMBER: 17283/117/GUPL

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)672-5300
(B) TELEFAX: (202)672-5399

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1343 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 27..1334

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Thr Leu Val Leu Val Ala Val Gly Pro Trp Val Leu Ser Ala Ala Ala
 10                  15                  20                  25

GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA GAG GTC GAC ATC ATA       149
Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val Glu Val Asp Ile Ile
                    30                  35                  40

GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG TCT GTC GGG       197
Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu Ser Val Gly
                45                  50                  55

AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT TGG       245
Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn Trp
            60                  65                  70

ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT       293
Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe
        75                  80                  85

TCT TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA       341
Ser Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg
 90                  95                  100                 105

GCA GAA AAA GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA       389
Ala Glu Lys Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr
                110                 115                 120

CCA TTT CGC AAA GCT CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT       437
Pro Phe Arg Lys Ala Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala
            125                 130                 135

GAA GAT AAG GCA ATA GTG ATA CAC ATC TCT CCT GGA ACA AAA GAT AGT       485
Glu Asp Lys Ala Ile Val Ile His Ile Ser Pro Gly Thr Lys Asp Ser
        140                 145                 150

GTT ATG TGG GCT TTG GAT GGT TTA AGC TTT ACA TAT AGC TTA CTT ATC       533
Val Met Trp Ala Leu Asp Gly Leu Ser Phe Thr Tyr Ser Leu Leu Ile
    155                 160                 165

TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT GAA AAT ATT TAT TCC       581
Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile Glu Asn Ile Tyr Ser
170                 175                 180                 185

AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT TGT CTA AAA       629
Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr Cys Leu Lys
                190                 195                 200

GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT CCA       677
Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser Pro
            205                 210                 215

GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA       725
Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu
        220                 225                 230

AAT ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT       773
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                270                 275                 280

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            285                 290                 295

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        300                 305                 310

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                350                 355                 360

ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT     1157
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            365                 370                 375

TCA AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT     1205
Ser Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val
        380                 385                 390

CCT AAT TTG AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC     1253
Pro Asn Leu Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His
    395                 400                 405

ACC ATG GAT GAA AAG CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA     1301
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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 65                  70                  75                  80

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                 85                  90                  95

Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys Glu Asn Thr Ser
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Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala Gln Ile
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145                 150                 155                 160

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        195                 200                 205

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    210                 215                 220

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            260                 265                 270

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        275                 280                 285

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    290                 295                 300

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305                 310                 315                 320

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                325                 330                 335

Phe Asn Ile Arg Ser Leu Ser Asp Ser Phe His Ile Tyr Ile Gly Ala
            340                 345                 350

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Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser Asn Ala Glu Arg Lys Ile
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Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu Lys Pro Leu Thr
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Asn Thr Ser Lys
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1755 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 27..1697

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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Thr Leu Val Leu Val Ala Val Gly Pro Trp Val Leu Ser Ala Ala Ala
 10                  15                  20                  25

GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA GAG GTC GAC ATC ATA       149
Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val Glu Val Asp Ile Ile
                30                  35                  40

GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG TCT GTC GGG       197
Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu Ser Val Gly
            45                  50                  55

AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT TGG       245
Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn Trp
        60                  65                  70

ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT       293
Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe
    75                  80                  85

TCT TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA       341
Ser Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg
 90                  95                  100                 105

GCA GAA AAA GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA       389
Ala Glu Lys Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr
                110                 115                 120

CCA TTT CGC AAA GCT CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT       437
Pro Phe Arg Lys Ala Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala
            125                 130                 135
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GAA GAT AAG GCA ATA GTG ATA CAC ATC TCT CCT GGA ACA AAA GAT AGT        485
Glu Asp Lys Ala Ile Val Ile His Ile Ser Pro Gly Thr Lys Asp Ser
        140                 145                 150

GTT ATG TGG GCT TTG GAT GGT TTA AGC TTT ACA TAT AGC TTA CTT ATC        533
Val Met Trp Ala Leu Asp Gly Leu Ser Phe Thr Tyr Ser Leu Leu Ile
    155                 160                 165

TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT GAA AAT ATT TAT TCC        581
Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile Glu Asn Ile Tyr Ser
170                 175                 180                 185

AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT TGT CTA AAA        629
Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr Cys Leu Lys
                190                 195                 200

GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT CCA        677
Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser Pro
            205                 210                 215

GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA        725
Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu
        220                 225                 230

AAT ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT        773
Asn Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp
    235                 240                 245

TAT ACA TAT GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT        821
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TTA AAA AGG AAT CCT GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT        869
Leu Lys Arg Asn Pro Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro
                270                 275                 280

GAC TGT GAA AAT GTC AAA ACT ACC CAG TGT GTC TTT CCT CAA AAC GTT        917
Asp Cys Glu Asn Val Lys Thr Thr Gln Cys Val Phe Pro Gln Asn Val
            285                 290                 295

TTC CAA AAA GGA ATT TAC CTT CTC CGC GTA CAA GCA TCT GAT GGA AAT        965
Phe Gln Lys Gly Ile Tyr Leu Leu Arg Val Gln Ala Ser Asp Gly Asn
        300                 305                 310

AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT GAT ACT GAA ATA CAA       1013
Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe Asp Thr Glu Ile Gln
    315                 320                 325

GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT AGT GAT TCA       1061
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330                 335                 340                 345

TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT GTG       1109
Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro Val
                350                 355                 360

ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT       1157
Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr
            365                 370                 375

TCA AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT       1205
Ser Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val
        380                 385                 390

CCT AAT TTG AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC       1253
Pro Asn Leu Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His
    395                 400                 405
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410                 415                 420                 425

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Cys Glu Lys Thr Lys Pro Gly Asn Thr Ser Lys Ile Trp Leu Ile Val
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GGA ATT TGT ATT GCA TTA TTT GCT CTC CCG TTT GTC ATT TAT GCT GCG      1397
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            445                 450                 455

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        460                 465                 470

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Pro Ser Ser Ser Ile Asp Glu Tyr Phe Ser Glu Gln Pro Leu Lys Asn
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490                 495                 500                 505

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                510                 515                 520

GAA GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT      1637
Glu Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn
            525                 530                 535

TAT TCT AAT GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG      1685
Tyr Ser Asn Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln
        540                 545                 550

CAG GAC TTT GTA TGACCAGAAA TGAACTGTGT CAAGTATAAG GTTTTTCAGC         1737
Gln Asp Phe Val
    555

AGGAGTTACA CTGGTACC                                                 1755
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 557 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Met Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val
 1               5                  10                  15

Gly Pro Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser
            20                  25                  30

Pro Gln Lys Val Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg
        35                  40                  45

Trp Asn Arg Ser Asp Glu Ser Val Gly Asn Val Thr Phe Ser Phe Asp
    50                  55                  60
```

```
Tyr Gln Lys Thr Gly Met Asp Asn Trp Ile Lys Leu Ser Gly Cys Gln
 65                  70                  75                  80

Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser Ser Leu Lys Leu Asn Val
                 85                  90                  95

Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys Glu Asn Thr Ser
            100                 105                 110

Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala Gln Ile
        115                 120                 125

Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile
    130                 135                 140

His Ile Ser Pro Gly Thr Lys Asp Ser Val Met Trp Ala Leu Asp Gly
145                 150                 155                 160

Leu Ser Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val
                165                 170                 175

Glu Glu Arg Ile Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu
            180                 185                 190

Ser Pro Glu Thr Thr Tyr Cys Leu Lys Val Lys Ala Ala Leu Leu Thr
        195                 200                 205

Ser Trp Lys Ile Gly Val Tyr Ser Pro Val His Cys Ile Lys Thr Thr
    210                 215                 220

Val Glu Asn Glu Leu Pro Pro Pro Glu Asn Ile Glu Val Ser Val Gln
225                 230                 235                 240

Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr Ala Asn Met Thr
                245                 250                 255

Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro Gly Asn
            260                 265                 270

His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr
        275                 280                 285

Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu
    290                 295                 300

Leu Arg Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu
305                 310                 315                 320

Glu Ile Lys Phe Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val
                325                 330                 335

Phe Asn Ile Arg Ser Leu Ser Asp Ser Phe His Ile Tyr Ile Gly Ala
            340                 345                 350

Pro Lys Gln Ser Gly Asn Thr Pro Val Ile Gln Asp Tyr Pro Leu Ile
        355                 360                 365

Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser Asn Ala Glu Arg Lys Ile
    370                 375                 380

Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu Lys Pro Leu Thr
385                 390                 395                 400

Val Tyr Cys Val Lys Ala Arg Ala His Thr Met Asp Glu Lys Leu Asn
                405                 410                 415
```

Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly
420 425 430

Asn Thr Ser Lys Ile Trp Leu Ile Val Gly Ile Cys Ile Ala Leu Phe
435 440 445

Ala Leu Pro Phe Val Ile Tyr Ala Ala Lys Val Phe Leu Arg Cys Ile
450 455 460

Asn Tyr Val Phe Phe Pro Ser Leu Lys Pro Ser Ser Ser Ile Asp Glu
465 470 475 480

Tyr Phe Ser Glu Gln Pro Leu Lys Asn Leu Leu Leu Ser Thr Ser Glu
485 490 495

Glu Gln Ile Glu Lys Cys Phe Ile Ile Glu Asn Ile Ser Thr Ile Ala
500 505 510

Thr Val Glu Glu Thr Asn Gln Thr Asp Glu Asp His Lys Lys Tyr Ser
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Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val
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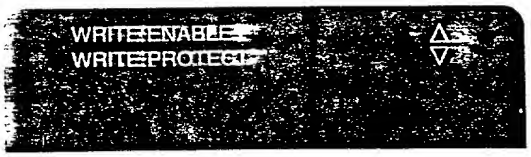

IBM PC AT 1.44mb MSDOS 3.3

~~BENOIT, et al.~~

US 08/307,588

FILED:05-DEC-1994

~~"MONOCLONAL ANTIBODIES~~

AGAINST THE INTERFERON..."

ATTY DOCKET:17283/117/GUPL

~~DATA REC'D:26 MAR 1996~~

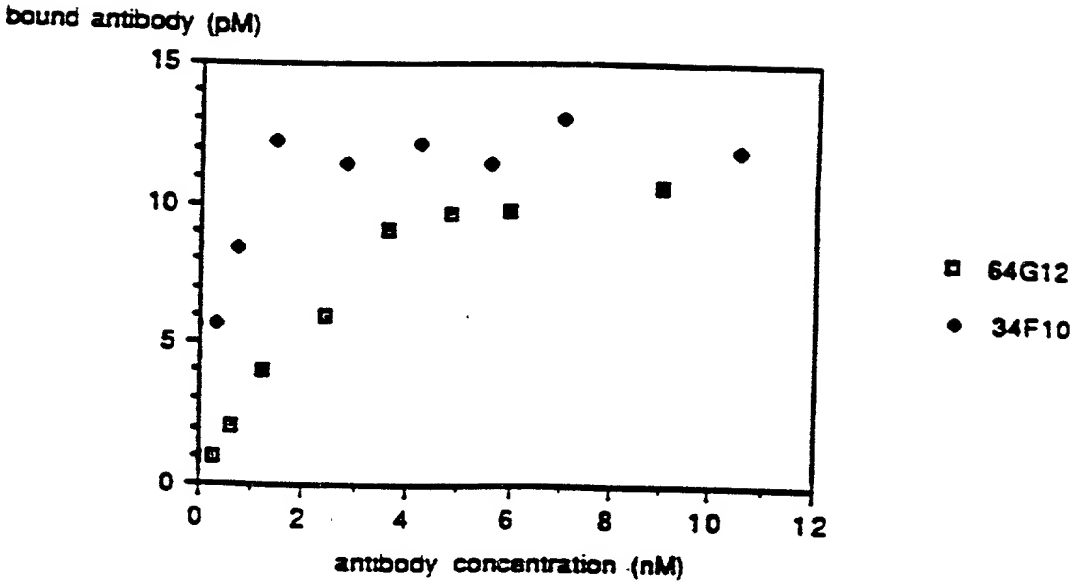

FIGURE 1A

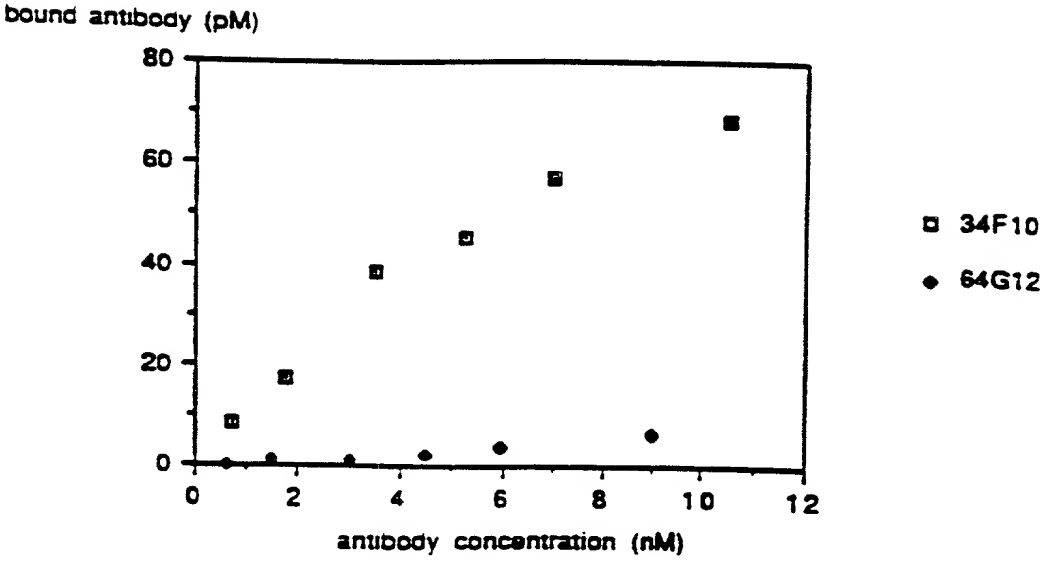

FIGURE 1B

CTGCAGGCATCTGCGGCGGCTCCCAG

```
ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
Met Met Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
His Ile Ser Pro Gly Thr Lys Asp Ser Val Met Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn Met Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu
```

FIGURE 2A

SUBSTITUTE SHEET

```
AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr Met Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGA GGT ACC
Asn Thr Ser Lys
```

FIGURE 2B

CTGCAGGCATCTGCGGCGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
Met Met Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
His Ile Ser Pro Gly Thr Lys Asp Ser Val Met Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn Met Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

FIGURE 3A

SUBSTITUTE SHEET

```
AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAC TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr Met Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA ATT TGG CTT ATA GTT GGA ATT TGT ATT GCA TTA TTT GCT CTC
Asn Thr Ser Lys Ile Trp Leu Ile Val Gly Ile Cys Ile Ala Leu Phe Ala Leu

CCG TTT GTC ATT TAT GCT GCG AAA GTC TTC TTG AGA TGC ATC AAT TAT GTC TTC
Pro Phe Val Ile Tyr Ala Ala Lys Val Phe Leu Arg Cys Ile Asn Tyr Val Phe

TTT CCA TCA CTT AAA CCT TCT TCC AGT ATA GAT GAG TAT TTC TCT GAA CAG CCA
Phe Pro Ser Leu Lys Pro Ser Ser Ser Ile Asp Glu Tyr Phe Ser Glu Gln Pro

TTG AAG AAT CTT CTG CTT TCA ACT TCT GAG GAA CAA ATC GAA AAA TGT TTC ATA
Leu Lys Asn Leu Leu Leu Ser Thr Ser Glu Glu Gln Ile Glu Lys Cys Phe Ile

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TGA
Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC
```

FIGURE 3B

Docket No. 17283/117/GUPL

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

the specification of which (check one)

☐ is attached hereto

☒ was filed on *March 30, 1993* as Application Serial No. *PCT/EP93/00770* and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	*European*	*31/March/1992*	*Yes*

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

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Address telephone communications to *Bernhard D. Saxe* at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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 Docket No. 17283/117/GUPL

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Post Office Address		
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Full Name of Second Inventor	Signature of Second Inventor	Date
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Full Name of Third Inventor	Signature of Third Inventor	Date
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Post Office Address *24, rue Maitre-Albert, F-75005 Paris, France*		

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Residence Address *Fresnes, France*	**Country of Citizenship** ~~*FRANCE*~~ CROATIA	
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Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Michael G. TOVEY	x [signature]	
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Post Office Address *6, rue des Quatrefages, F-75005 Paris, France*		